Review

Total Synthesis and Biological Activity of Lactacystin, Omuralide and Analogs

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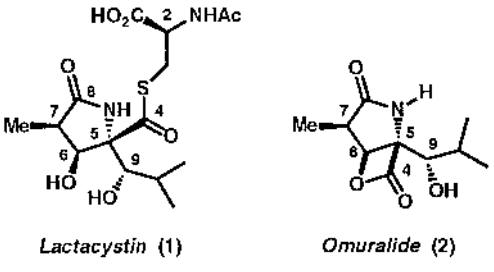
Lactacystin (1) and the related β-lactone omuralide (2) are remarkably selective and potent irreversible inhibitors of the 20*S* proteasome, a large polymolecular protein machine which is responsible for the degradation of ubiquitin-labeled proteins. Because of this fact 1 and 2 have emerged as important tools in biochemistry and cell biology. The challenge of synthesis has been accepted by several research groups with the result that 1 and 2 and their analogs can now be synthesized by a variety of synthetic approaches. This review summarizes the synthetic processes which have been developed to date for the production of such compounds. The study of biological activity of analogs of 1 and 2 has clearly defined the structural features which are responsible for the potency of 1 and 2, as described in the closing section of this account. It is concluded that 1 and 2 are nearly optimal for the irreversible inactivation of the 20*S* proteasome.

Key words lactacystin; omuralide; synthesis; bioactivity; proteasome; inhibition

Lactacystin (**1**), a structurally novel microbial product, was isolated from a *Streptomyces* strain and characterized by Ōmura *et al*.[1,2] from a screening program to detect nerve growth factor (NGF)-like activity (induction of neurite outgrowth) in a mouse neuroblastoma cell line (Neuro 2A). The structure was unequivocally determined as **1** by X-ray crystallographic studies.[2] A detailed investigation of the mode of biological action of lactacystin revealed that it did not function as a mimic of nerve growth factor.[3,4] Instead, lactacystin has been found to act with exquisite specificity and in a unique fashion to inhibit irreversibly the proteolytic activity of the 20*S* proteasome, a cylindrical complex of 28 protein subunits which is responsible for the hydrolytic fragmentation of ubiquitinated proteins. The thiol ester function of lactacystin is sufficiently reactive to allow spontaneous conversion to the β-lactone **2**[5] which similarly deactivates the 20*S* proteasome, *but at a much faster rate*.[4,5] The major source of inactivation of the 20*S* proteasome appears to be the acylation of the *N*-terminal threonine subunit, a key participant in proteolytic catalysis,[3,4] to form inactivated proteasome as shown in Fig. 1. This result was confirmed by X-ray crystallographic studies of the lactacystin-inactivated 20*S* proteasome at 2.4 Å resolution.[6,7] Because the proteasome machinery is involved in the degradation of many proteins, including not only misfolded and denatured molecules[8] but also proteins involved in cell cycle progression[9] and regulation of gene transcription,[10] lactacystin has emerged as a very important new tool for the study of protein biochemistry and cell biology.[11,12]

The key evidence which defined the mechanism of action of lactacystin may be summarized as follows. (1) Incubation of Neuro-2A cells with C(9) tritiated lactacystin (from chemical synthesis[3]) labels with extraordinary selectivity a protein which was identified as a proteasome β-subunit by acetylation of the *N*-terminal threonine.[3] (2) The inactivation of the proteasome occurs in a *time-dependent* and *irreversible* manner by either lactacystin (**1**) or lactacystin β-lactone (**2**),[5] with the latter being much more reactive. (3) Lactacystin derivatives which can not form a β-lactone are inactive.[4] (4) A variety of ester analogs of lactacystin which are sufficiently reactive to form spontaneously the β-lactone **2**, *e.g.* thiol esters or the 2,2,2-trifluoroethyl ester, are active.[4] (5) Lactacystin β-lactone (**2**) is formed from lactacystin in extracellular fluid and diffuses more readily through the cell membrane.[13] Because of the biological importance of **2** and the desirability of giving it a sample name, we have chosen to call it "omuralide" in this review. Lactacystin (**1**) and omuralide (**2**) are remarkable because they exemplify dramatically the ability of small molecules to shut down the functioning of a very large poly-macromolecular machine and to exert this inhibition with great selectivity on 20*S* proteasome in the presence of countless other proteins as potential targets. The biological uniqueness and utility of lactacystin and omuralide and their structural novelty have made these molecules and their analogs attractive targets for chemical synthesis. This report reviews the studies carried out thus far in this



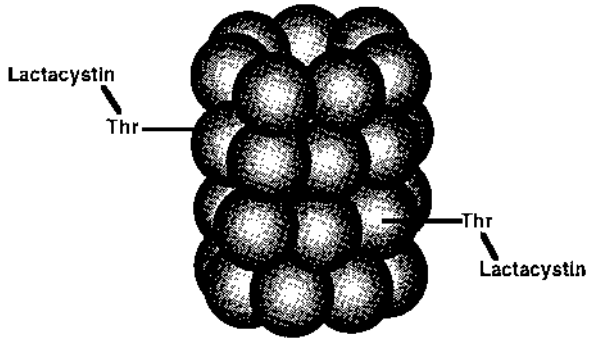


Fig. 1. 20*S* Proteasome after Irreversible Inhibition by Lactacystin (**1**) or **2**

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field. It also summarized the current state of knowledge regarding the relationship of biological activity to chemical structure.

Initial Studies on the Total Synthesis of Lactacystin

The first total synthesis of lactacystin[14] and omuralide and refinements subsequently made to it[15] are summarized in Chart 1. The protected aminal **4**, which was obtained from (*S*)-serine (**3**) in three standard steps, was converted diastereoselectively to the crystalline product **5** *via* the corresponding lithium enolate by reaction with isobutyraldehyde. The presence of added lithium bromide was required for diastereoselectivity. Acidic methanolysis of the aminal subunit in **5**, silylation of the resulting primary alcohol function, formation of an oxazoline ring by methylene connection of secondary hydroxyl and amino functions, reduction of methoxycarbonyl group by $LiBH_4$ and modified Swern oxidation generated aldehyde **6**. In the Swern oxidation step, it was advantageous to use a new modification in which the cold preformed Swern reagent (from Me_2SO and oxalyl chloride at $-78\,^{\circ}C$ in CH_2Cl_2) was added to a mixture of primary alcohol and Et_3N in CH_2Cl_2 solution at $-78\,^{\circ}C$. The conventional Swern oxidation procedure leads to inefficient formation of aldehyde **6** along with several by-products.

The transformation of aldehyde **6** to the *anti* aldol product **8** was one of the most challenging step in the synthesis. Initial approaches using Heathcock's methodology[14] (lithium enolate of 2,6-dimethylphenyl propionate) or Braun's approach[16,17] provided only moderate yields of the desired *anti* aldol product, and were unsatisfactory for use on larger scale.[15] As outlined in Chart 1, the Mukaiyama aldol coupling of **6** and **7** with MgI_2 as catalyst proceeded stereoselectively and in good yield, with no scale-up problems, to afford the aldol product **8**. It is noteworthy that MgI_2 was uniquely effective as catalyst.[15] For example, use of $SnCl_4$, $ZnCl_2$ or $Cu(OTf)_2$ in CH_2Cl_2 for the Mukaiyama aldol coupling of **6** and **7** produced none of the desired product **8**.[15] The unique effectiveness of MgI_2 as catalyst in the reaction **6**+**7**→**8** may be due to the ability of the small IMg^+ species to form a chelate with the sterically congested amino aldehyde **6** as shown in Chart 2.[15] Because of steric screening at the *re* face of the formyl group this chelate undergoes nucleophilic attack at the *si* face. Preferential formation of the *anti* aldol product (*anti*/*syn* ratio *ca*. 10 : 1), appears also to be due to steric effects which favor the *synclinal* transition state over the *antiperiplannar* alternative as shown in Chart 2.[15]

The key aldol intermediate **8** was transformed into the dihydroxy lactam **9** by the sequence: (1) *N*-benzyl cleavage, (2) amino ester→γ-lactam ring closure and (3) desilylation, as shown in Chart 1. Oxidation of the primary alcohol function of **9** produced the dihydroxy acid **10** which underwent selective β-lactonization to omuralide (**2**) when treated with bis(2-oxo-3-oxazolidenyl)phosphinic chloride (BOPCl) and Et_3N. Finally, reaction of omuralide (**2**) with *N*-acetyl-(*S*)-cysteine produced lactacystin (**1**) quantitatively. The process has been used successfully to produce multigram amounts of these compounds. At present it appears to be the most effective synthesis of **1** and **2** in quantity. Very little chromatography is involved in this synthesis. In addition, the conversions **5**→**6**, **8**→**9** and **9**→**10** can be carried out without purification of intermediates.[15] The initial aldol product **5** has been made on 100 g scale using only ordinary laboratory glassware and the conversion to lactacystin and omuralide can be accomplished in 40% overall yield.

The intermediates provided by the synthetic approach summarized in Chart 1 were also useful in providing analogs of lactacystin which are incapable of leading to β-lactone analogs of **2**. These structures were obviously useful as tests of the hypothesis that the formation of omuralide might be a prerequisite for lactacystin function. As shown in Chart 3, the *syn* aldol diastereomer **11** of the *anti* aldol intermediate **8** could be converted *via* the lactam **12** to the (6*R*,7*S*)-diastereomer (**13**) of lactacystin by a sequence of reactions paralleling those used for the synthesis of lactacystin from **8** (Chart 1).[18] Intermediate **12** also served admirably for the synthesis of the 6-deoxy analog (**14**) of lactacystin. Both analogs **13** and **14** of lactacystin were found to be biologically inactive which underscored the possibility that the omuralide might

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Wei-Dong Z. Li was born in Gansu, China in 1968. He received his Ph.D. degree in 1993 from Lanzhou University, China in organic chemistry for work on the synthesis of cembrane diterpenoids under the supervision of professor Yu-Lin Li. After a short period of stay at Lanzhou University, he became a postdoctoral research fellow (1995—1998) at Harvard University with professor E. J. Corey working on the synthesis and bioactivity of lactacystin. In 1998, he returned to China and currently is a Professor of Chemistry at the National Laboratory of Applied Organic Chemistry and the Institute of Organic Chemistry, Lanzhou University.

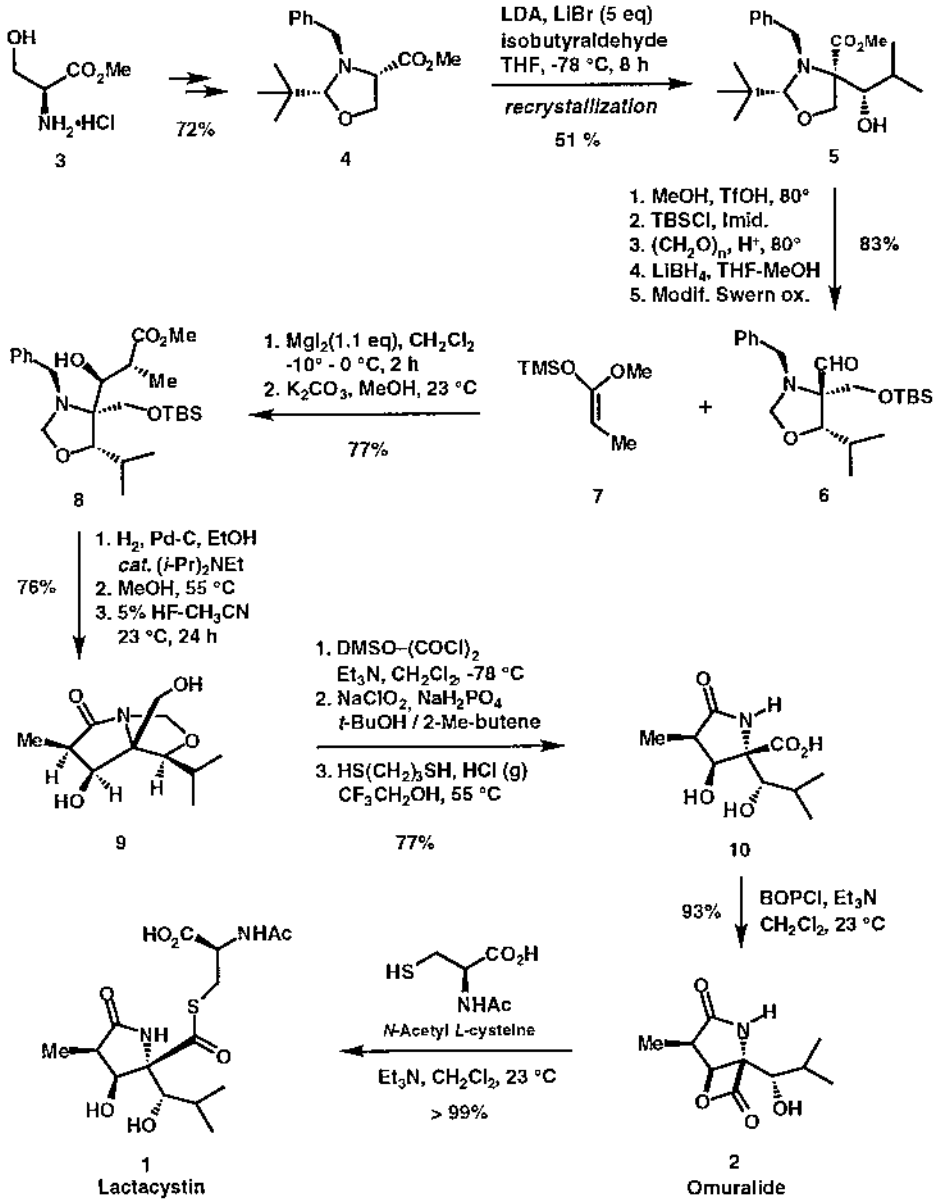


Chart 1. Efficient Total Synthesis of Lactacystin and Omuralide from (*S*)-Serine

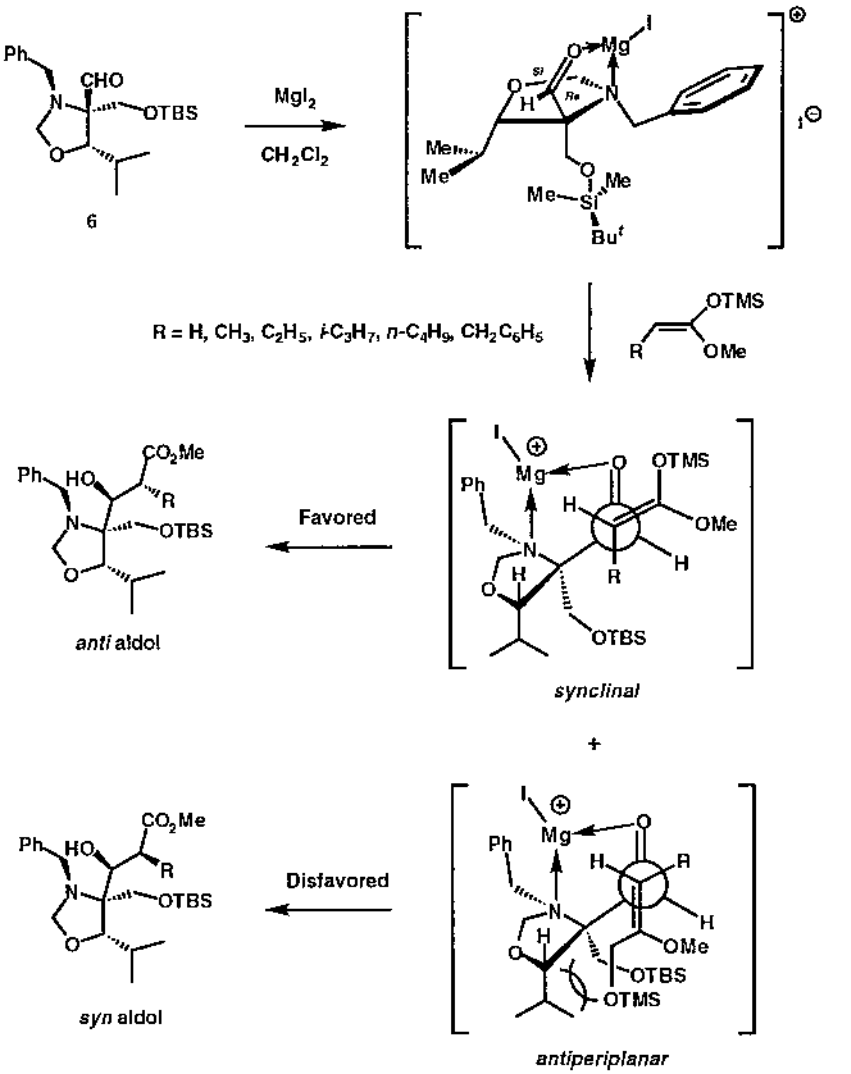


Chart 2. Doubly Diastereoselective Mukaiyama Aldol Coupling

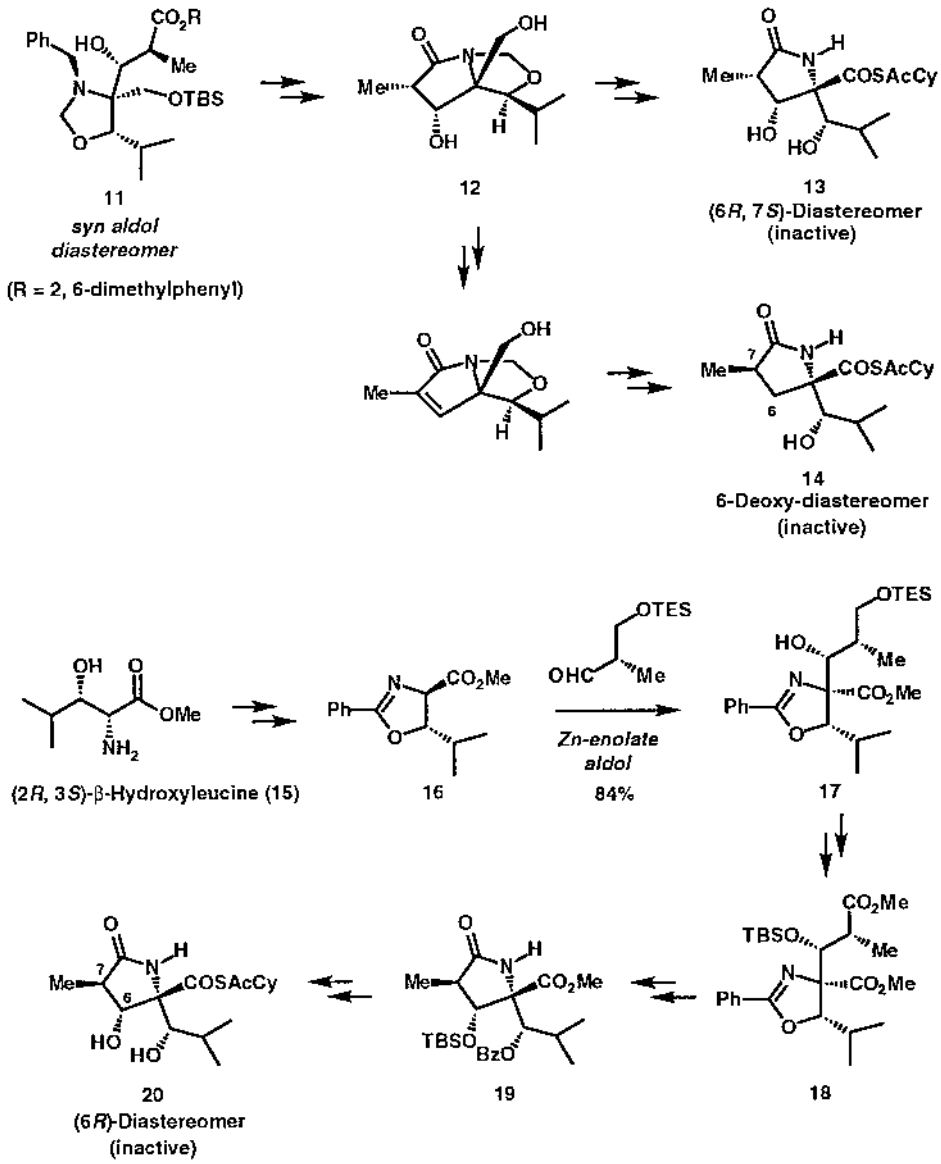


Chart 3. Synthesis of Lactacystin Analogs Incapable of Forming β-Lactone

be essential for the bioactivity of lactacystin.[4] Because of these results, a synthesis of the (6*R*)-diastereomer of lactacystin was developed as outlined in Chart 3. (2*R*,3*S*)-β-Hydroxyleucine (**15**) was prepared by a catalytic enantioselective route and converted to the oxazoline **16**. Aldol reaction of the zinc enolate of **16** with (*S*)-2-methyl-3-triethylsilyloxypropionaldehyde resulted in doubly diastereoselective formation of the *anti* aldol product **17**. The (6*R*)-diastereomer of lactacystin was synthesized from **17** *via* the oxazoline **18** and the lactam **19** as outlined in Chart 3. Diastereomer **20** was also shown to be devoid of lactacystin-like activity.[4]

(2*R*,3*S*)-β-Hydroxyleucine (**15**) and oxazoline **16** were utilized for a total synthesis of lactacystin by Ōmura, Smith and collaborators using an approach outlined in Chart 4.[20,21] Key steps in this synthesis include diastereoselective hydroxymethylation of **16** and oxidation to **21** and crotylboration of the labile aldehyde **21**.

Lactacystin has also been synthesized by Baldwin and coworkers starting with the (*R*)-glutamic acid-derived intermediate **23** which has the γ-lactam ring of lactacystin already in place, along with an extra stereocenter for stereochemical control (Chart 5).[22,23] α-Methylation of **23** and introduction of α,β-unsaturation provided **24** which was silylated to the interesting chiral pyrrole **25**. Mukaiyama aldol reaction of **25** with isobutyraldehyde produced the required coupling product **26** in modest yield. Installation of the β-hydroxy lactam subunit required a number of steps which led to the hydroxy lactam **27**, and finally to **1**, as shown in Chart 5.[22,23]

Quite a different synthesis of **1** was developed by Chida *et al.* using D-glucose as starting material.[24,25] This synthesis, though long, has several interesting features, as shown in Chart 6. Only four of the six carbons and one of the five stereocenters of D-glucose survive in the final product. C(4) of D-Glucose becomes C(7) of lactacystin. The C(5) quaternary stereocenter of **1** is created with 4 : 1 diastereoselectivity by Overman [3, 3] sigmatropic rearrangement (**28**$\rightarrow$**29**). The generation of the C(9) stereocenter (**30**$\rightarrow$**31**) suffered from lack of stereocontrol and low yield due to concomitant formyl reduction by isopropylmagnesium bromide.[24,25]

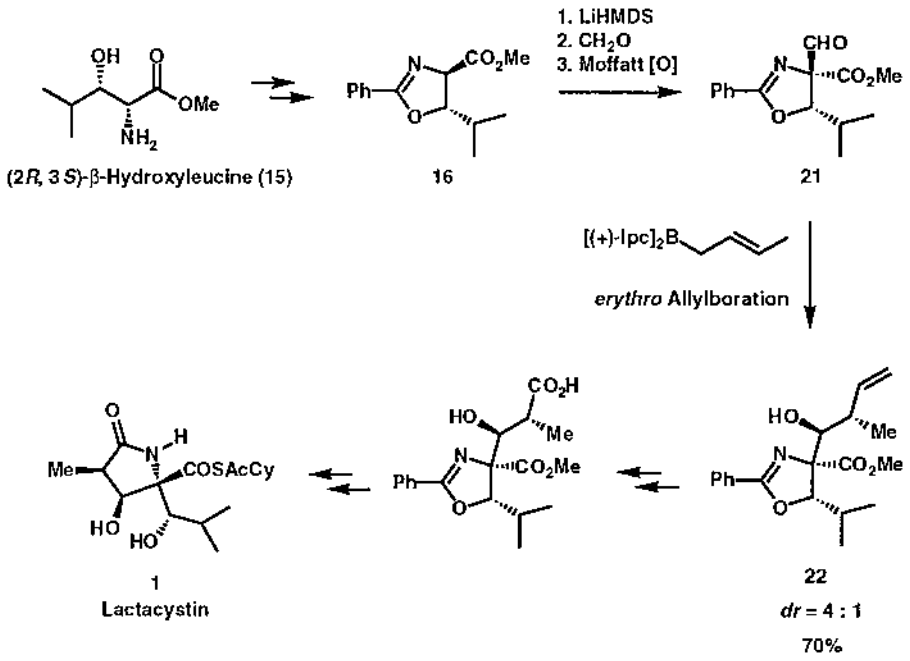


Chart 4. Omura–Smith Synthesis of Lactacystin

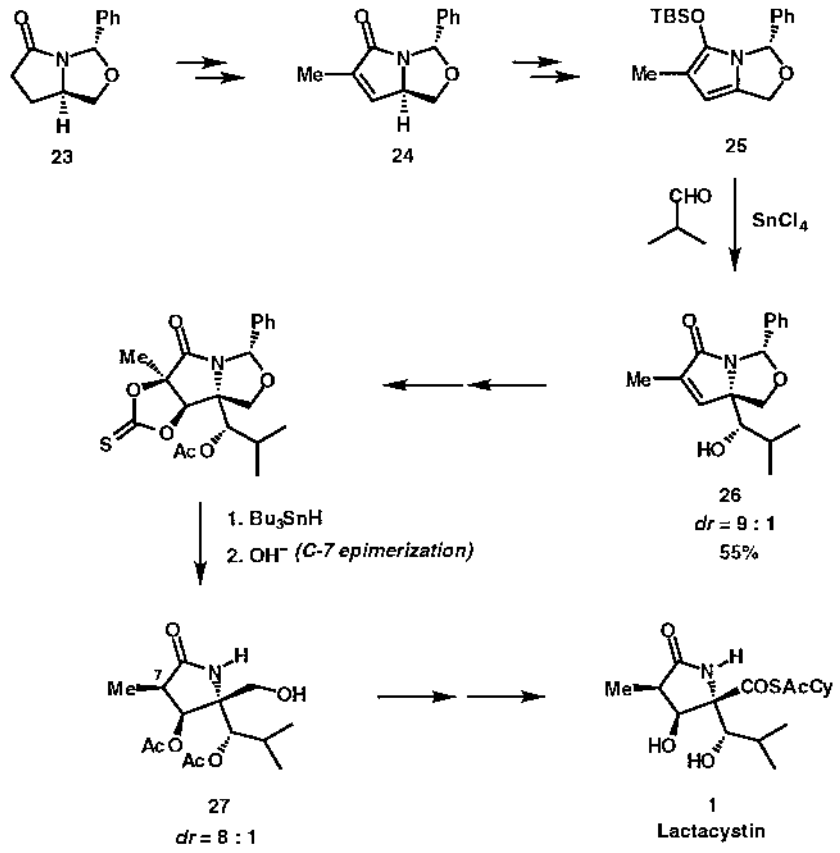


Chart 5. Baldwin Synthesis of Lactacystin

The first enantioselective synthesis of lactacystin from achiral compounds, dimethyl malonate derivative **28** and methyl *N*-*p*-methoxybenzylglycinate, is summarized in Chart 7.[26] Enantioselective pig liver esterase (PLE)-catalyzed hydrolysis followed by one recrystallization of the resulting monoacid as the quinine salt afforded the chiral acid **29** (*S* configuration, 95% ee) which was coupled to the glycine ester component and cyclized to give the chiral keto lactam **30**. Hydroxymethylation of **30** and reduction of the ketonic carbonyl by internal hydroxyl-directed hydride delivery each

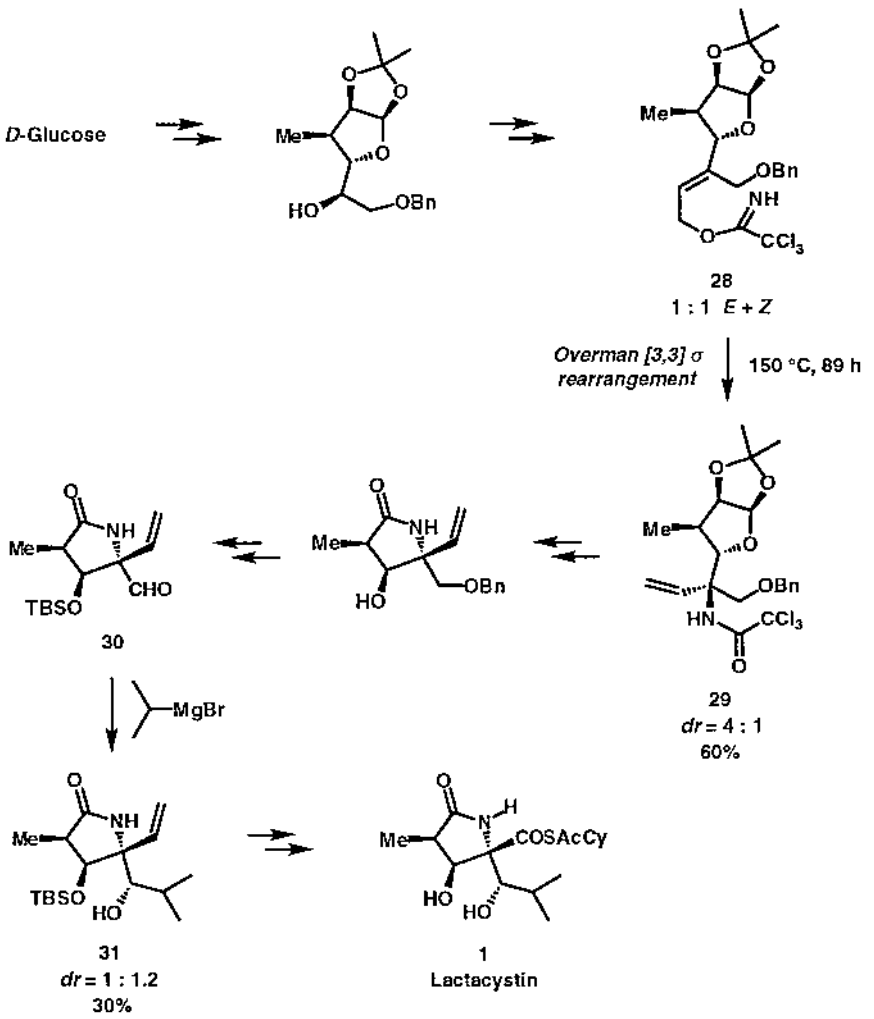


Chart 6. Chida Synthesis of Lactacystin

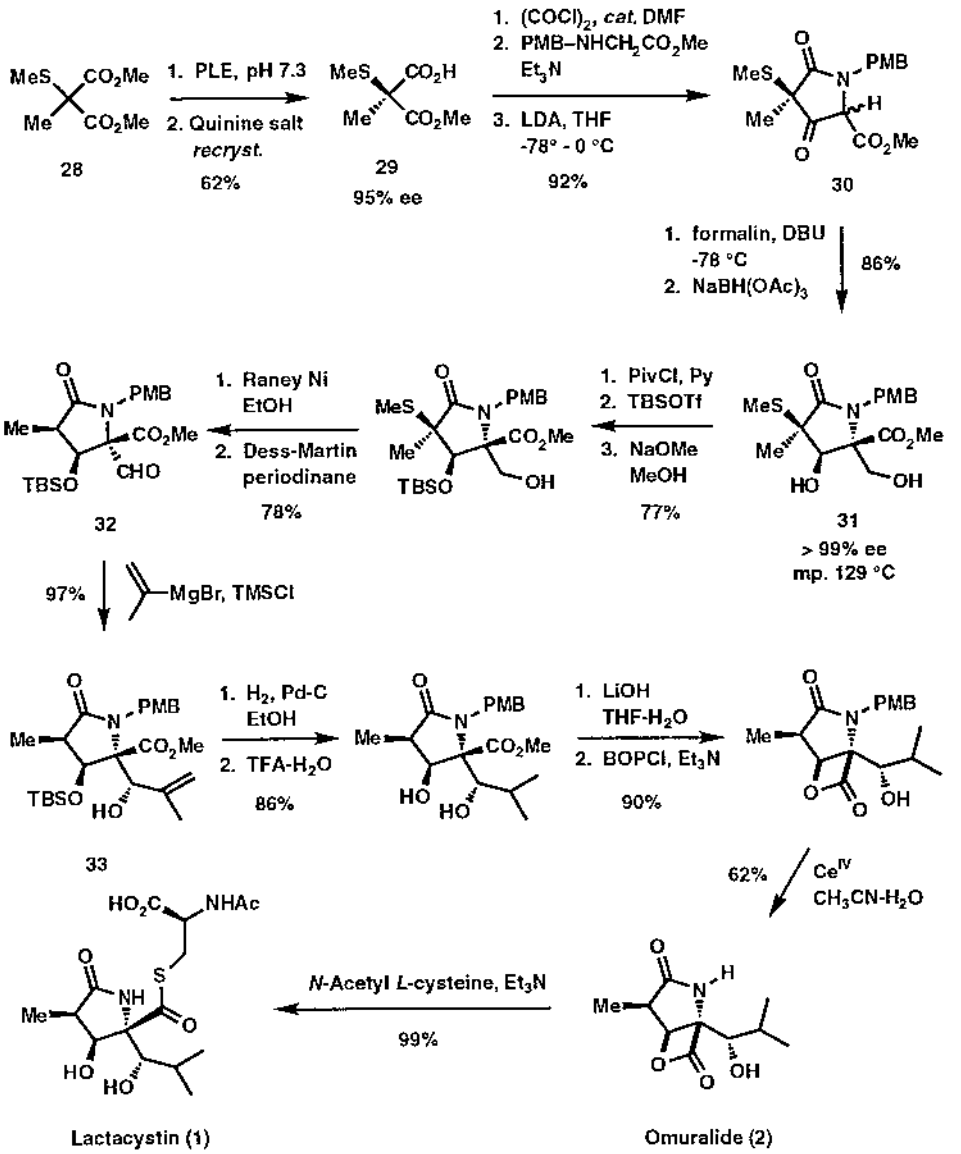


Chart 7. Enantioselective Total Synthesis of Lactacystin and Omuralide from Achiral Components

Table 1. Kinetics of Inhibition of the Chymotrypsin-Like Peptidase Activity of Purified 20 *S* Proteasome (from Bovine Brain[3]) by β-Lactone Analogs of Lactacystin: $K_{assoc.}=K_{obs}/[I]$ ($M^{-1}\cdot s^{-1}$) (Triplicate Runs)

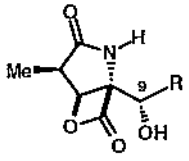
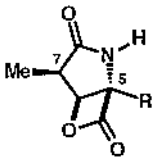
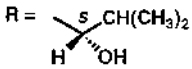
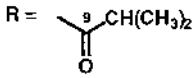
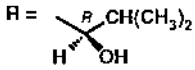
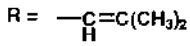
Analog structure	$K_{assoc.}$ ($M^{-1}\cdot s^{-1}$)
 **34**	
R = $-CH(CH_3)_2$ (2)	3059 ± 478
R = –H (35)	9.7 ± 6.2
R = $-C_6H_5$ (36)	no inhibition
R = $-C_2H_5$ (37)	290 ± 12
R = $-CH=CH_2$ (38)	188 ± 11
R = $-CH_2CH_2CH_3$ (39)	192 ± 35
R = $-CH_2CH=CH_2$ (40)	255 ± 40
R = $-CH_2CH(CH_3)_2$ (41)	17.4 ± 2.4
R = $-CH_2C(CH_3)=CH_2$ (42)	64.7 ± 2.2

Table 2. Kinetics of Inhibition of the Chymotrypsin-Like Peptidase Activity of Purified 20 *S* Proteasome (from Bovine Brain[3]) by β-Lactone Analogs of Lactacystin: $K_{assoc.}=K_{obs}/[I]$ ($M^{-1}\cdot s^{-1}$) (Triplicate Runs)

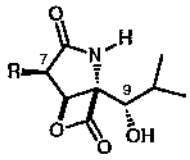
Analog structure	$K_{assoc.}$ ($M^{-1}\cdot s^{-1}$)
	
R =  (2)	3059 ± 478
R =  (43)	no inhibition
R =  (44)	65 ± 9.6
R = $-CH_2CH(CH_3)_2$ (45)	235 ± 16
R =  (46)	98 ± 5

occurred with high diastereoselectivity to form **31** (99% ee after one recrystallization), the structure of which was confirmed by X-ray crystallographic analysis.[26] After conversion of **31** to the aldehyde **32**, reaction of **32** with the 2-propenyl Grignard reagent in the presence of trimethylchlorosilane (essential to avoid *retroaldol* cleavage) provided the required adduct **33** in high yield. High diastereoselectivity was again observed for the sequence **31** → **32** → **33**. Intermediate **33** was transformed into **1** and **2** by standard methods.[26] The synthesis outlined in Chart 7 constitutes not only an efficient and practical route to lactacystin and omuralide, but also an excellent approach to the synthesis of numerous analogs in which the isopropyl group at C(9) in **1** and **2** is replaced by other substituents.

Synthesis and Biological Activity of Analogs of Lactacystin (1) and Omuralide (2)

The remarkable potency and specificity of lactacystin (**1**) and the omuralide (**2**) in proteasome inactivation raised the interesting questions of whether these compounds had been optimized during evolution for this purpose and whether they could be improved upon. As indicated in the preceding paragraph the synthetic approach outlined in Chart 7 provided the intermediate **32** from which a variety of C(9) substituents can be introduced to generate β-lactone analogs of type **34**. By this means were synthesized not only **34**, R = CH=CH_2 and R = $C(CH_3)$=CH_2 and their dihydro derivatives but also **34**, R = C_6H_5. In addition, **34**, R = CH_2CH=CH_2 and R = $CH_2C(CH_3)$=CH_2 were prepared by the allylation of aldehyde **32** using the appropriate allyl bromide, manganese metal, and $CrCl_3$ as catalyst in the presence of trimethylchlorosilane,[27] and these products were converted in turn to the corresponding analogs with saturated groups at C(9).[28] The biological activity of this series of analogs, including **34**, R = H was assessed relative to omuralide by measurement of inactivation rate constants of purified bovine brain 20 *S* proteasome.

Table 3. Kinetics of Inhibition of the Chymotrypsin-Like Peptidase Activity of Purified 20 *S* Proteasome (from Bovine Brain[3]) by β-Lactone Analogs of Lactacystin: $K_{assoc.}=K_{obs}/[I]$ ($M^{-1}\cdot s^{-1}$) (Triplicate Runs)

Analog structure	$K_{assoc.}$ ($M^{-1}\cdot s^{-1}$)
	
R = CH_3– (2)	3059 ± 478
R = H– (47)	450 ± 77
R = CH_3CH_2– (48)	6679 ± 553
R = $CH_3(CH_2)_3$– (49)	7275 ± 486
R = $(CH_3)_2CH$– (50)	8465 ± 1572
R = $C_6H_5CH_2$– (51)	2227 ± 180
7-*epi*-Me (52)	1250 ± 66

The results of the kinetic measurements are summarized in Table 1. Clearly, the most active compound is omuralide (**2**). Replacement of the C(9)-isopropyl group of **2** by hydrogen (as in **35**) greatly reduces activity and substitution by phenyl (as in **36**) abolishes proteasome inhibition. Similarly, activity relative to **2** is greatly diminished with C(9) substituents which are either slightly *smaller* than isopropyl [*e.g.* ethyl (**37**) or vinyl (**38**)] or *larger* than isopropyl [(*e.g.* allyl (**40**),

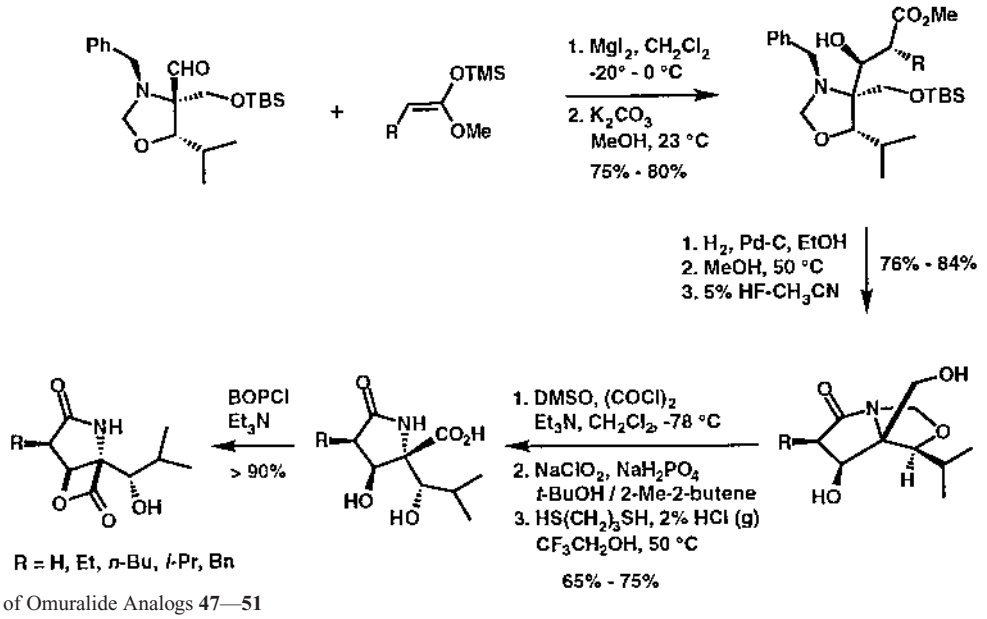


Chart 8. Synthesis of Omuralide Analogs **47**—**51**

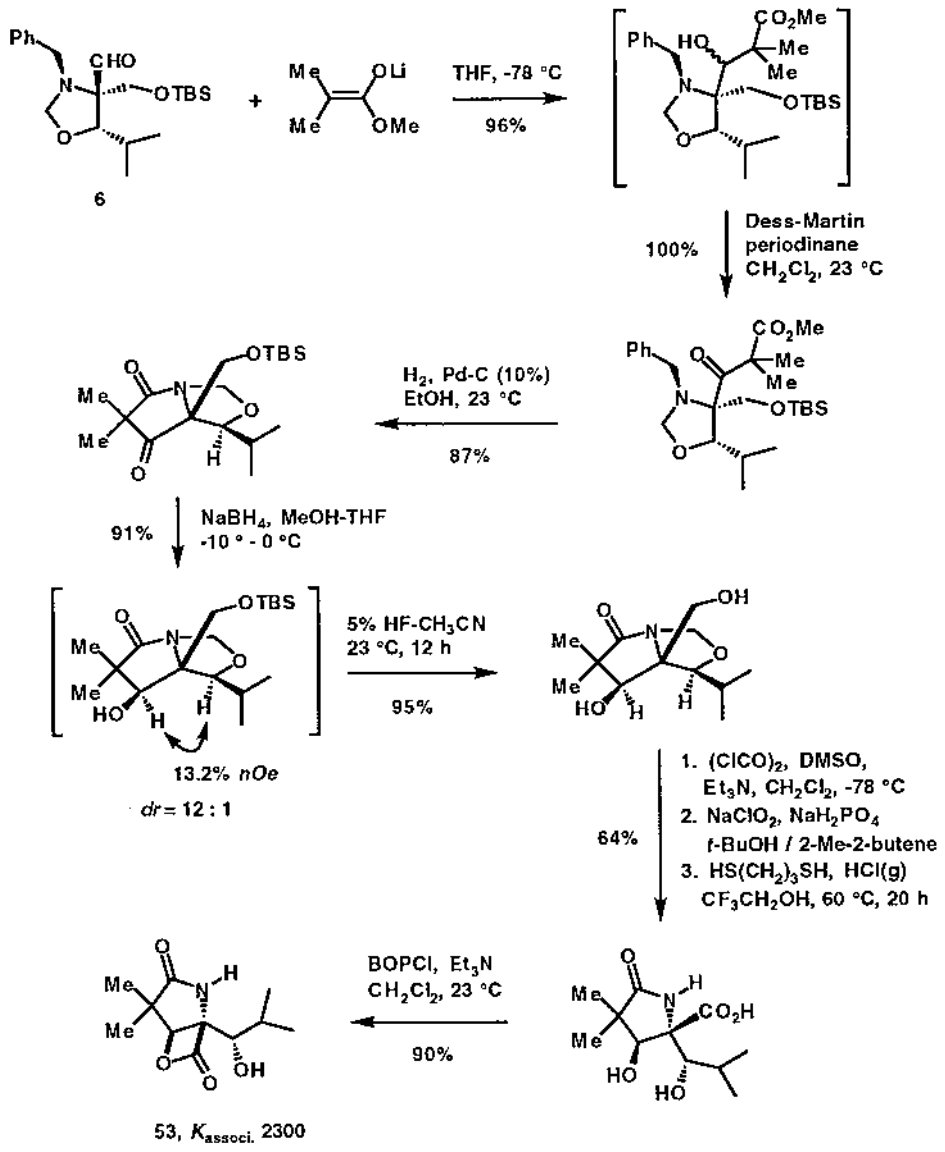


Chart 9. Synthesis of 7-Methyl-omuralide Analog (**53**)

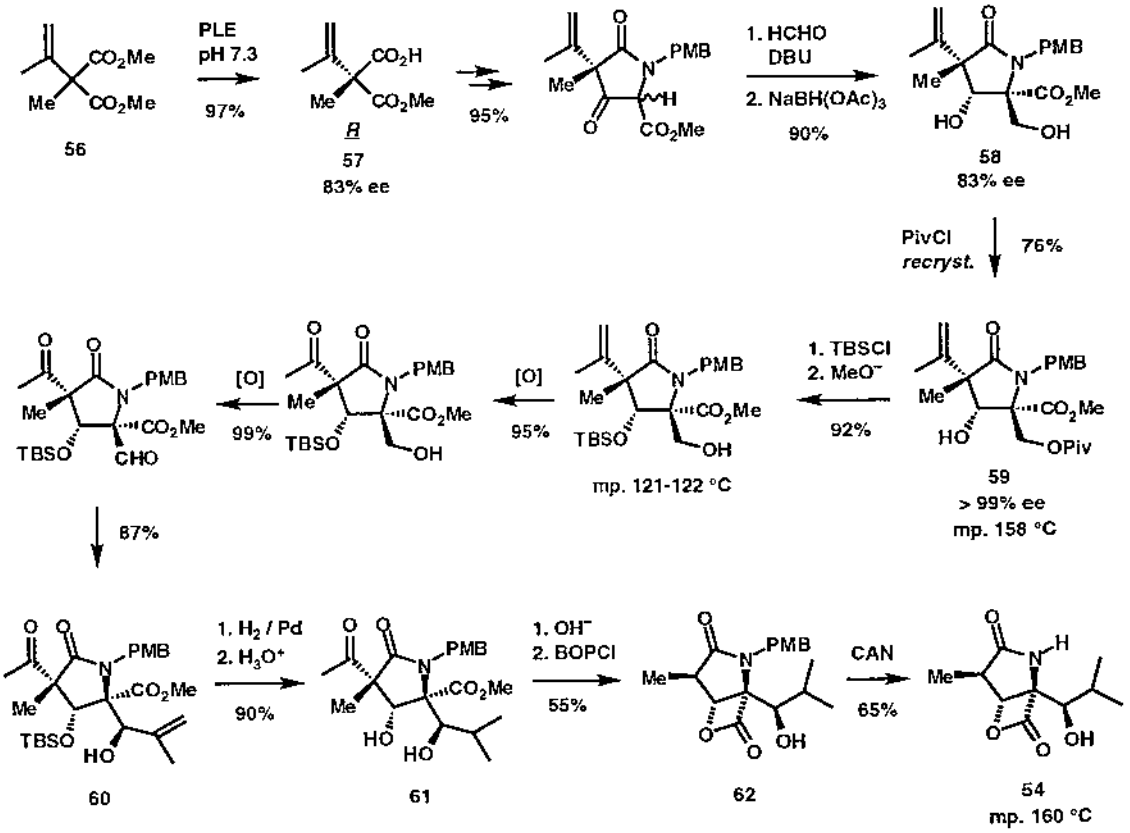


Chart 10. Enantioselective Synthesis of (5*S*,6*R*,9*R*/*S*)-Diastereomers **54** and **55** of Omuralide

n-propyl (**39**), methallyl (**42**) or isobutyl (**41**)]. Thus, it seems clear that the C(9)-isopropyl substituent of the natural products **1** and **2** is optimal for mammalian proteasome inhibition, implying a fairly snug fit for isopropyl in the complementary binding pocket of the proteasome.

Previous biological studies established the importance of C(5)-α-hydroxyisobutyl sidechain for bioactivity.[4a] In addition, it was shown that there must be a hydroxyl group at C(6) *cis* to the C(4)-carboxylic group (*i.e.* suitably placed for β-lactone formation); 6-deoxy- and 6(*R*)-hydroxy analogs of lactacystin were completely inactive in proteasome inhibition. Similar studies were carried out with regard to the C(9)-hydroxyl function as summarized in Table 2. Analogs **43** and **44** were prepared as previously described.[3] Analog **45** was prepared by catalytic hydrogenation of **46** (H_2, Pd–C) which was synthesized from aldehyde **32** by the sequence (1) Wittig reaction with $(CH_3)_2C{=}PPh_3$, (2) ester hydrolysis, (3) β-lactonization, and (4) *N*-deprotection, essentially using the methodology outlined in Chart 7. Comparison of the data in Table 2 reveals that the functionality and configuration at C(9) of **2** are critical to proteasome inhibition and that the omuralide structure **2** is optimal with respect to bioactivity.

We have also investigated the relationship between the nature of the substitution at C(7) in analogs of omuralide and ability to inactivate the mammalian 20*S* proteasome. The analogs which were prepared and tested (**47**—**52**) are shown in Table 3. The synthesis of these compounds was accomplished by the methodology described in Chart 1, as summarized in Chart 8.[15] The data in Table 3 indicate that whereas the replacement of the C(7)-methyl group of omuralide by the smaller hydrogen leads to much reduced activity (**47**), replacement of the C(7) methyl group by the larger substituents ethyl (**48**), *n*-butyl (**49**), or isopropyl (**50**) results in an approximate doubling of the proteasome inhibitory activity relative to **2**. The C(7)-diastereomer of **2** (**52**) and the C(7)-benzyl analog (**51**) are somewhat less active, but still potent. Thus, in the case of the C(7)-substituent the activity of omuralide, while high, is not quite optimum.

The synthesis of an analog of omuralide having an additional methyl substituent at C(7) (**53**) was accomplished expeditiously as outlined in Chart 9.[29] This synthesis is both diastereocontrolled and practical. The product **53** is most interesting since it is almost as active as omuralide ($K_{assoc.}$ for **53**, 2302 $M^{-1}\cdot s^{-1}$).

The effect of changing the stereochemical orientation of the β-lactone bridge on the γ-lactam nucleus has also been determined by the synthesis of β-lactones **54**, the (5*S*,6*R*,9*R*)-diastereomer of omuralide, and **55**, the (5*S*,6*R*,9*S*)-diastereomer of omuralide. The synthetic pathway to **54** is summarized in Chart 10.[30] Enantioselective hydrolysis of **56** gave **57** (*R* configuration, 83% ee)[30] which was transformed by a reaction sequence paralleling that shown in Chart 7 to diol **58** and pivolate ester **59**, which could be recrystallized to enantiomeric purity.

Conversion of **59** to **60** and deacetylation (LiOH, 80 °C) of **61**, as shown, provided **62** which was deprotected to **54**. The C(9)-diastereomer (**55**) of **54** was prepared similarly from the C(9)-diastereomer of **60** (a minor byproduct in the Grignard addition to form **60**). The relative rates of proteasome inactivation by omuralide, **54** and **55** were found to be 3060, 121

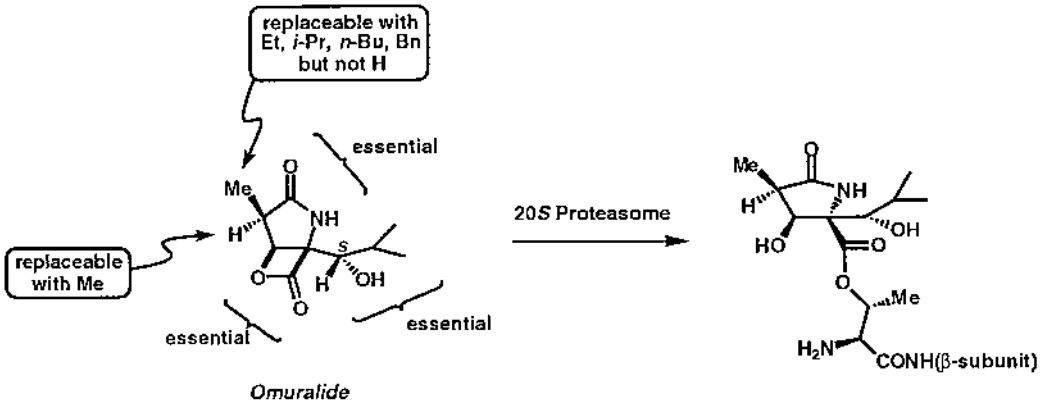


Fig. 2. Summary of Structure–Activity Studies

and 0, respectively, a clear indication that the correct *stereo-orientation* of the β-lactone bridge of omuralide is critical to bioactivity.

Figure 2 summarizes all the results obtained thus far on the biological activity of lactacystin/omuralide and analogs.

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